Fat pad alterations with ageing and osteoarthritis in an Ovine model

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Published in:
Osteoarthritis and Cartilage

DOI:
10.1016/j.joca.2023.01.341

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

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A WHOLE-JOINT HISTOPATHOLOGIC GRADING SCHEME FOR MURINE KNEE OSTEOARTHRITIS

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Purpose: Reproducible and reliable histologic assessments of osteoarthritis (OA) severity in animal models are critical for evaluating the efficacy of therapeutic agents designed to prevent or attenuate the disease and for comparing results among researchers using standardized grading criteria. Several semi-quantitative histologic OA grading systems have been created to assess OA severity in humans and animal models. These systems have laid solid foundations with valuable methodologies for OA grading. Nevertheless, specific limitations remain to be addressed. One of the limitations is that the most widely used histologic OA grading systems for humans and mice mainly focus on articular cartilage while scoring methods for periarticular tissues are either undefined or recommended with subjective scoring categories (e.g., mild, moderate, and severe). This study aims to develop a comprehensive and easily executable histopathologic grading scheme for murine knee OA using specific scoring criteria for both cartilage and periarticular changes, which may overcome important limitations of the existing grading systems.
Methods: The new grading scheme was developed based on mouse knee OA models with observation periods up to 24 months of age (spontaneous OA) or 24-week post-injury (posttraumatic OA). Semi-quantitative assessments of the histopathologic OA changes were applied to all four quadrants per femorotibial joint for 50 joints (200 quadrants) using specific scoring criteria rather than mild to severe grades. Scoring elements per quadrant were as follows: cartilage lesion (0-7), osteophyte (0-3), subchondral bone change (0-3), synovitis (0-3), and ectopic periarticular soft tissue chondrogenesis and ossification (0-3).
Results: The new histopathologic grading scheme had high intra- and inter-observer reproducibility (correlation coefficients $r > 0.95$) across experienced and novice observers. Sensitivity and reliability analyses confirmed the ability of the new scheme to detect minimal but significant OA progression ($p < 0.01$) within a two-week interval and to accurately identify tissue- and quadrant-specific OA severity within the joints.
Conclusions: This study presents the first whole-joint histopathologic grading scheme for murine knee OA that covers all-stage osteoarthritic changes in all major joint tissues, including periarticular soft tissue ossification that is not included in any of the existing OA grading systems. This reproducible scheme is easy to execute and sensitive to minimal OA progression without using computer software, suitable for quick OA severity assessments of the entire femorotibial joint.

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DEVELOPING AND CHARACTERIZING AN OSTEOCHONDRAL-SYNOVIAL MODEL FOR EVALUATING PSORIATIC ARTHRITIS THERAPIES

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Purpose: The overall purpose of this study is to develop and characterize a human osteochondral-synovial model for evaluating psoriatic arthritis (PsA) therapies. Two aims of the study; Aim 1: Establish and optimize a local end-stage PsA-like model based on a validated human OA model. Aim 2: Preliminary validation of the local end-stage PsA-like model by adding dexamethasone (DEX), a known anti-inflammatory drug, to the co-culture.
Methods: Synovial fluid is obtained from PsA patients and tissues from knee joints following total knee replacement surgery from OA patients. A metal punch with a diameter of 2.7mm is used to obtain biopsies of the cartilage-bone tissues, and the synovium tissues are minced into 1mmx1mm pieces. After 48 hours of acclimatization, the tissues are co-cultured in a 24-well plate. There is one negative control, including tissues + media and one positive control, including the tissues + 10ng/mL IL-1ß and 10ng/mL TNFα. In addition, there are also four treatment groups, including the tissues+ OA SF±DEX and the tissues+ PsA SF±DEX. After 7 days and 14 days of co-culture, the explant tissues were snap-frozen. The RNA isolation is done on snap-frozen tissues, and the real-time quantitative polymerase chain reaction is done to investigate the gene expression of the cartilage-bone and synovium explant tissues.
Results: The addition of PsA SF to the explant tissues changed the expression of the cartilage-bone and synovium but not in a dose-dependent matter. In addition, Investigating the gene expression of genes such as MMP1, MMP3, ADAMTS5, IL-6, and CXCL8 after adding the PsA SF showed us that the PsA SF was able to shift the gene expression profile of the explant tissues towards a more pro-inflammatory and degradative profile compared to when OA SF was added. There was also a rescue effect when dexamethasone was added to both PsA SF and OA SF treatments.
Conclusions: We have developed an end-stage joint-specific PsA-like model and showed that our model is sensitive enough to capture changes in the explant tissues when anti-inflammatory drugs such as dexamethasone are added.

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FAT PAD ALTERATIONS WITH AGEING AND OSTEOARTHRITIS IN AN OVINE MODEL

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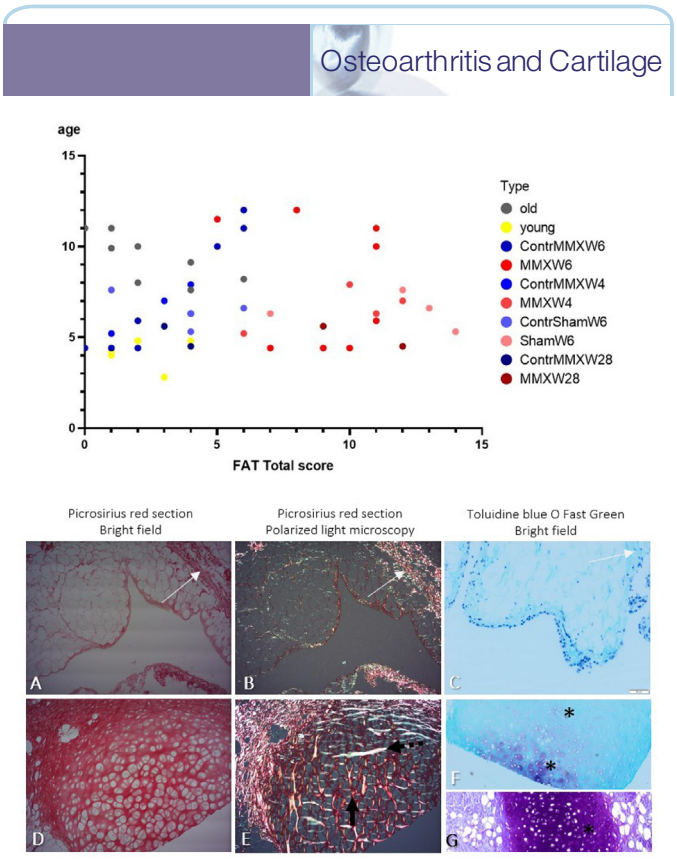
Purpose: The infrapatellar fat pad (IFP, Hoffa fat pad) within the knee joint has been described, over the last 20 years, as space-filler, source of inflammatory cytokines, shock absorber, as well as source of mesenchymal stromal cells. The IFP and the synovium might play a role in the pathogenesis of osteoarthritis (OA). In the current study, we aimed to characterize IFP alterations in ageing healthy sheep and in research sheep after meniscectomy and cranial cruciate transection.
Methods: Sixteen healthy ewes ($n = 16$) (7 young adults, and 9 old adults, age cut-off 7 years-old) were euthanatized to assess the age-related changes of the fat pad. Synovial fluid was collected and assessed for volume, viscosity, colour and turbidity. Macroscopic and microscopic characteristics of synovium were assessed according to OARSI guidelines. Histological scoring of IFP on HES slices included: intimal hyperplasia (0-3), inflammatory cells infiltration (0-3), fat/fibrous tissue ratio (0-5), vascularity (0-3), with a total score from 0 (normal) to 14 (severe hyperplasia of the intima, cell infiltration, vascularization and more than 80% of fibrous tissue (fat/fibrous tissue ratio). Resection of the cranial part of the medial meniscus and transection of the cranial cruciate ligament were performed in 16 other ewes ($n = 16$) whilst only skin incision (sham surgery) was performed in 4 ewes ($n = 4$). Synovial fluid was collected at baseline. Animals were euthanized at different time points, i.e. 4 ($n = 4$), 6 ($n = 8$) and 28-weeks ($n = 4$), and macroscopic and microscopic assessment was performed on the synovium, the cartilage and bone according to OARSI guidelines. Fat pad changes were further documented with Toluidine Blue O Fast Green staining (to detect proteoglycan deposit), and Picrosirius Red staining (observed under polarized light microscopy, to assess collagen fibres organization).
Results: Synovial fluid from all young adult and old adult sheep was normal (viscous, clear, and transparent). Superficial roughening of the cartilage of the medial femoro-tibial compartment was associated with ageing and present in sheep after 7 years. After meniscectomy and cruciate ligament transection, moderate synovial fluid alteration was identified, as well as moderate to severe changes in synovium and mild to moderate changes in fat pad (discoloration, hypervascularity). Sham surgery led to slighter changes. Multivariate analysis revealed that score for synovium assessment, at histology, was influenced by the type of intervention (non-operated controlateral, sham, PTOA induction; $P=0.02$) but not by the age of the sheep ($P=0.30$). Scoring of fat pad at histology was influenced by age of the animal ($P=0.03$) and the type of intervention ($P=0.003$). Histological changes of fad pad included interstitial fibrosis (2 out of 7 young adults, 6 out of 9 old adults, 10 of 16 operated sheep, and 2 out of 4 sham sheep). Chondroid metaplasia was found in 6 old adults and in 6 operated sheep, and was observed as



dense conjunctive tissue, with cells trapped inside lacunae, surrounded by organized collagen (picrosirius red) and partial blue staining (toluidine Blue). Correlation between fat pad scoring and synovium scoring was moderate (95% CI r=0.45-0.79, P<0.001).
Conclusions: Although considered as a space-filler or a cushion, the fat pad showed changes associated with ageing and surgery (partial medial meniscectomy and cranial cruciate ligament transection) in the sheep. Those changes (fibrosis, hypervascularization, cartilaginous metaplasia) could be involved in the pathogenesis of OA and alter the biomechanics and the secretory properties of the fat pad.
Legend: Graph: Total score at histology, for fat pad from young adults (yellow), old adults (grey), limbs after surgery (red dots, 4-, 6- or 28-weeks after PTOA induction or sham operated) and contralateral non-operated limbs (blue dots). Histology of normal fat pad (A-C) and fat pad with cartilaginous metaplasia (D-G). A-C: normal fat pad (white arrow) is featured by small amount of interstitial fibres (white arrows). D: In fat pad with cartilaginous metaplasia, lacunae are visible on Picrosirius red sections, under light microscope. E: Under polarized light microscope, the collagen fibres show some degree of alignment, shown as homogenous colour (vertical-red or horizontal-blue). F-G: Various toluidine blue intake (*) suggests different degree of metaplasia and proteoglycan deposition.

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ASSOCIATION OF SEMIMEMBRANOSUS TENDINOPATHY, PES ANSERINE BURSOPATHY AND MEDIAL GASTROCNEMIUS PATHOLOGY IN PATIENTS WITH MEDIAL KNEE PAIN: ANALYSIS OF DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Purpose: To determine if medial knee pain was associated with pathology of the distal semimembranosus tendon, pes anserine bursa, or proximal medial gastrocnemius muscle in subjects who had or were at elevated risk for developing knee osteoarthritis.
Methods: A cross-sectional case-control design was used to study 61 randomly selected participants, with knee pain over the past 7 days, medial joint line and pes anserine bursa tenderness in the absence of lateral joint line and patellofemoral tenderness (cases) and 23 control participants with knee pain over the last 7 days but without medial joint line or pes anserine tenderness from the Osteoarthritis Initiative (OAI) database at the 48-month clinic visit. MRIs were read for the presence or absence of T2 hyperintensity or thickness greater than 5 mm indicating semimembranosus tendinopathy, T2 hyperintensity within the pes anserine bursa indicating pes anserine bursopathy, and T2 hyperintensity within the proximal medial gastrocnemius muscle. The prevalence of each of these findings was compared with case-control status using Wald Chi Squared tests or, when appropriate, Fisher's exact test.
Results: Participants were predominantly female (67%) and white (88%) with an average age of 63.7±9.2 years. 62.3% of cases and 34.8% of controls had one or more soft tissue MRI findings (p=0.02). Prevalence of the 3 MRI findings were higher in cases than controls as follows: semimembranosus tendinopathy (31.1% of cases and 17.4% of controls, p=0.21), pes anserine bursopathy (27.9% of cases and 13.0% of controls, p=0.16), and medial gastrocnemius pathology (48.8% of cases and 13.0% of controls, p=0.07).
Conclusions: Previous studies have revealed that intra-articular knee imaging findings in patients with knee osteoarthritis do not always correlate with pain. Here, extra-articular soft-tissue pathology (semimembranosus tendinopathy, pes anserine bursopathy, or medial gastrocnemius pathology) tended to be more prevalent in people with medial knee pain and positive physical examination findings compared to those without medial knee pain. Extra-articular structures should be considered as pain generators in patients with medial knee pain and physical examination findings.

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RESPONSE OF KNEE CARTILAGE T2 RELAXATION TIMES TO 21-DAYS OF 6° HEAD-DOWN-TILT BED REST COMBINED WITH EXERCISE AND NUTRITION COUNTERMEASURES

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Purpose: While mechanical stress is known to influence the regulation of musculoskeletal tissue metabolism, the effects of immobilization on articular cartilage health in healthy individuals are not well understood. Magnetic resonance imaging (MRI) T2 relaxation times (T2Me) of articular cartilage are qualitative measures of cartilage quality where longer T2Me corresponds to poorer tissue quality. This study aims to investigate the effects of serial 21-days of 6° head-down-tilt bed rest (HDT-BR) periods combined with nutrition and exercise countermeasures on T2Me of the tibiofemoral articular cartilage in healthy male individuals.

Table 1: Descriptive statistics, Mean (Standard deviation) of T2 relaxation times per compartment by campaign and time points. MT – medial tibia, MF – medial femur, LT – lateral tibia, cLF – central lateral femur, D – deep, S – superficial, BDC – baseline data collection, HDT – head down tilt, R – recovery, T2Me - T2 relaxation times.

	Campaign 1			Campaign 2			Campaign 3		
Parameters	BDC-7	HDT21	R+6	BDC-7	HDT21	R+6	BDC-7	HDT21	R+6
Medial Tibia									
MTDT2Me	47.6(3.4)	49.3(6.0)	50.1(3.5)	50.1(2.4)	50.8(3.5)	48.1(2.9)	50.7(3.7)	51.4(5.9)	48.6(2.2)
MTST2Me	54.1(3.1)	54.5(4.8)	54.0(3.1)	54.5(3.2)	55.2(2.9)	53.4(2.3)	54.5(2.7)	55.1(4.1)	53.3(3.4)
Central Medial Femur									
cMFDT2Me	45.2(2.6)	46.6(5.5)	47.5(4.1)	47.1(3.1)	48.2(3.7)	45.7(2.4)	47.2(3.8)	48.1(5.1)	45.8(2.2)
cMFST2Me	57.2(4.6)	57.0(5.5)	56.4(4.0)	57.8(4.1)	56.8(3.3)	56.1(4.0)	57.7(4.6)	57.8(5.8)	56.9(5.1)
Lateral Tibia									
LTDT2Me	50.2(2.7)	51.5(7.1)	53.2(3.8)	50.3(2.9)	50.9(4.4)	49.8(2.2)	50.0(4.7)	50.8(7.6)	48.4(2.9)
LTST2Me	52.2(2.3)	53.4(5.6)	53.6(1.6)	52.5(2.5)	53.2(2.0)	52.5(2.2)	53.5(2.4)	53.3(3.4)	52.4(2.0)
Central Lateral Femur									
cLFDT2Me	44.7(2.3)	46.3(4.7)	47.1(2.4)	45.9(2.5)	47.3(2.1)	45.7(2.2)	46.4(2.5)	45.7(4.1)	45.9(2.2)
cLFST2Me	57.7(5.1)	59.1(5.6)	58.8(4.5)	58.8(5.2)	58.1(4.5)	58.0(5.2)	58.7(5.8)	58.7(6.6)	58.6(5.2)